RESEARCH PAPER

Formulation and Antimicrobial Evaluation of Herbal Facewash Gels Containing Colchicine and Barbaloin Against Propionibacterium acnes with Optimization of Active Ingredient Ratios

Gaurav Kela[1], Mohini Kuchekar[1*], Bhushan Pimple[1], Prasad Kadam[2], Kavita Yadav[2], Abhijit Karanje[2], Minal Harde[3], Omprakash Swami[4]

[1]Department of Pharmacognosy, Progressive Education Society's Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India-411044

[2]Department of Pharmacognosy, Marathwada Mitra Mandal College of Pharmacy, Thergaon, Pune, Maharashtra, India-411033

[3]Department of Pharmachemistry, Progressive Education Society's Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India-411044

[4]Department of Quality Assurance, Progressive Education Society's Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India-411044

Corresponding Author:

Dr. Mohini C. Kuchekar

Department of Pharmacognosy, Progressive Education Society's Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India-411044

Email: mohini.kuchekar@gmail.com

ABSTRACT

Background: *Acne vulgaris*, primarily caused by *Propionibacterium acnes* colonization, affects a significant global population across adolescent and young adult age groups. Synthetic anti-acne formulations, while therapeutically effective, carry documented adverse effects including skin irritation, over-drying, hypersensitivity reactions, and disruption of skin barrier function. This investigation developed and evaluated herbal facewash gel formulations using natural phytoconstituents (colchicine, barbaloin, green tea extract, caffeine) as safer alternatives to conventional synthetic anti-acne products.

Material and Methods: Four individual herbal facewash gel formulations were prepared incorporating colchicine, barbaloin, green tea extract, and caffeine as active antimicrobial agents. Three additional combination formulations were developed employing colchicine and barbaloin in ratios of 1:1, 7:3, and 3:7. Comprehensive physicochemical characterization was conducted including pH determination, surface tension measurement, viscosity assessment, spreadability evaluation, foam production, foam stability testing, and sag/rinsability assessment. Antimicrobial activity was quantitatively evaluated against *Propionibacterium acne* (MTCC-1951) using agar well diffusion methodology at concentrations of 5%, 10%, 20%, and 40% (v/v).

Results: All individual herbal formulations exhibited physicochemical properties comparable to marketed anti-acne products, with pH values within the physiologically appropriate range (5.02–5.34), surface tension values of 34.2–38.59 dyne/cm, and viscosity ranging from 400–5500 cPas. Antimicrobial evaluation demonstrated colchicine and barbaloin as significantly superior anti-acne agents compared to green tea extract and caffeine. Critically, the colchicine: barbaloin formulation in 3:7 ratio demonstrated statistically superior antimicrobial activity against *P. acnes*, evidenced by significantly larger inhibition zones and highest R^2 values compared to 1:1 and 7:3 ratios and individual components.

Conclusions: This study successfully developed herbal facewash gels with optimized colchicine: barbaloin ratio (3:7) demonstrating superior anti-acne efficacy while maintaining acceptable physicochemical properties suitable for topical application. These formulations represent a scientifically validated alternative to synthetic anti-acne products, fulfilling emerging consumer demand for natural, safer plant-derived skin care therapeutics. The identified optimal formulation ratio provides a foundation for subsequent clinical efficacy studies in acne patient populations.

Keywords: Anti-acne; Antimicrobial evaluation; Barbaloin; Colchicine; Herbal facewash gel;*Propionibacterium acnes*.

How to cite this article: Kela G, Kuchekar M, Pimple B, Kadam P, Yadav K, Karanje A, Harde M, Swami O. Formulation and Antimicrobial Evaluation of Herbal Facewash Gels Containing Colchicine and Barbaloin Against Propionibacterium acnes with Optimization of Active Ingredient Ratios. Int J Drug Deliv Technol. 2026;16(6s): 538-547; DOI: 10.25258/ijddt.16.6s.78

INTRODUCTION

Acne vulgaris is one of the most common chronic inflammatory disorders, affecting a substantial proportion of the global population across adolescence and early adulthood. Epidemiological data indicate that the prevalence peaks during the teenage years, but clinically significant disease frequently persists into the third decade of life, remaining a concern for both males and females aged 11–30 years. The visible and often recalcitrant nature of acne lesions can markedly impair quality of life, self-esteem, and psychosocial functioning, underscoring the need for effective, well-tolerated, therapeutic and preventive strategies [1].

Pathophysiologically, *acne vulgaris* arises from a multifactorial process centered on the pilosebaceous unit. Key events include follicular hyperkeratinization, obstruction of the pilosebaceous duct, increased sebum production, colonization by Propionibacterium acnes and subsequent inflammatory responses. Hormonal fluctuations, particularly increased androgen levels, play a critical role by stimulating sebaceous gland activity and sebum secretion, thereby aggravating comedone formation and creating a lipid-rich environment that favors bacterial overgrowth and follicular inflammation [2]. These mechanistic insights highlight that both microbial control and appropriate cleansing of the follicular openings are important components of acne management.

Facial cleansers and facewash formulations constitute a fundamental part of daily acne care regimens. An appropriately designed facewash facilitates the removal of make-up residues, desquamated corneocytes, excess sebum, environmental pollutants, and other impurities from the skin surface. By promoting deep cleansing and effective pore decongestion, facewashes can reduce follicular blockage and, consequently, the risk of lesion development. Contemporary facewash products are available in multiple categories—herbal and synthetic, medicated, and non-medicated—and are often positioned as adjuncts to pharmacological acne therapy. However, synthetic facewashes based on harsh surfactant systems and chemical additives have been associated with undesirable effects such as excessive dryness, hypersensitivity reactions, irritation, and disruption of the skin barrier and microbiome [3].

In contrast, herbal facewashes employ plant-derived extracts and phytoconstituents that may offer multiple benefits, including hydration, improvement of skin texture, prevention of breakouts, gentle cleansing, and anti-inflammatory or antimicrobial effects. The shift towards herbal cosmetics reflects growing consumer awareness of the potential risks associated with synthetic ingredients and a preference for “natural” alternatives. The global herbal cosmetics market has expanded rapidly , within this context, *acne vulgaris*—often driven by follicular blockage and inflammation—remains a key indication for which herbal facewash formulations are increasingly being sought and used [4].

Numerous herbal active agents have been reported to possess anti-acne or antimicrobial activity; however, there is still a need for systematically developed formulations that combine scientifically selected phytoconstituents with favorable physicochemical properties and demonstrable efficacy. The present work focuses on four such natural actives: colchicine, barbaloin, green tea extract, and caffeine. These phytoconstituents were selected on the basis of literature reports of antimicrobial and/or anti-inflammatory effects relevant to acne, along with their good aqueous solubility at room temperature and proven compatibility with commonly used gel-forming and surfactant excipients [5].

Accordingly, the aim of this study was to formulate and evaluate herbal anti-acne facewash gels containing colchicine, barbaloin, green tea extract, and caffeine, and to further investigate combinations of the most promising actives. The specific objectives were to develop physically and cosmetically acceptable formulations, to characterize their key physicochemical and performance parameters, and to assess their in vitro antimicrobial activity against Propionibacterium acnes in order to identify optimized herbal facewash compositions with potential utility as safer, plant-based alternatives to conventional synthetic anti-acne cleansers.

2. MATERIALS AND METHODS

2.1. Materials

Green tea leaves were procured from local suppliers in Pune, India. Colchicine, caffeine, and barbaloin

(purity ≥98%) were obtained from Yucca Laboratory Pvt Ltd, Mumbai. The bacterial strain *Propionibacterium acnes* (MTCC-1951) was acquired from the Gene Bank and Microbial Type Culture Collection (IMTECH), Chandigarh, India. Pharmaceutical excipients including Carbopol 934, triethanolamine, sodium lauryl sulfate, methyl paraben, rose oil, glycerin, and tocopherol (vitamin E) were sourced from Research-Lab Fine Chemicals Industries, Mumbai. All chemicals used were of analytical or pharmaceutical grade unless otherwise specified. Ultrapure water (Milli-Q system) was used throughout the study.

2.2. Preparation of Green Tea Extract

Green tea extract was prepared using a standardized hot water extraction method. Briefly, dried green tea leaves (1 kg) were extracted with ultrapure water (5 L) at temperatures maintained between 85–95°C for 30 minutes with continuous stirring. The resulting suspension was concentrated to 20% (w/v) using a rotary evaporator under reduced pressure at 50°C. The concentrated extract was subsequently filtered through a 10-micron polypropylene filter bag and further concentrated to yield approximately 5.0% solid content. The extract was stored at 4°C in amber-colored glass bottles until further use. The total yield and extraction efficiency were calculated gravimetrically.

2.3. Formulation of Herbal Facewash

The facewash formulation was prepared using a two-phase mixing protocol:

Phase A (Gel phase): Carbopol 934 (1.5% w/v) was dispersed in ultrapure water under continuous stirring at room temperature (25 ± 2°C) using a bath sonicator (frequency: 40 kHz, duration: 15 minutes) to achieve complete hydration. Triethanolamine was subsequently added drop-wise to neutralize the polymer and form a clear gel matrix. Phase B (Surfactant phase): Sodium lauryl sulfate (3% w/v), methyl paraben (0.2% w/v), glycerin (5% w/v), rose oil (0.5% v/v), vitamin E (0.3% w/v), and the green tea extract (varying concentrations) were dissolved in ultrapure water with gentle heating (40–45°C) and continuous stirring for 10 minutes until a homogeneous suspension was obtained. Final formulation: Phase B was slowly added to Phase A under controlled mixing (100 rpm) using a mechanical stirrer for 20 minutes at room temperature to ensure uniform distribution. The final formulation was transferred to sterile containers and aged for 24 hours at room temperature prior to evaluation Table 1. All formulations were prepared in triplicate.

2.4. Physicochemical Characterization

2.4.1. Visual Inspection

All prepared facewash formulations were visually inspected for physical appearance, including color, odor, clarity, and the presence of any particulate matter or phase separation.

2.4.2. Surface Tension Measurement

The density of 10% (v/v) facewash solutions was determined at 25 ± 1°C using a calibrated pycnometer (volume: 25 mL). The same samples were subsequently transferred to a calibrated stalagmometer for surface tension determination using the standard drop-count method [6]. All measurements were performed in triplicate, and the surface tension was calculated using the equation: $\sigma = (\rho \times g \times V) / (n \times 10)$, where ρ is the density, g is acceleration due to gravity, V is the volume of liquid, and n is the number of drops.

2.4.3. pH Determination

All facewash formulations were diluted to 10% (v/v) in ultrapure water. The pH was measured using a calibrated digital pH meter (accuracy ± 0.01 pH units) at 25 ± 1°C. The electrode was calibrated using buffer solutions of pH 4.0 and 7.0 (phosphate buffers) immediately prior to each set of measurements. Each formulation was analyzed in triplicate [7].

2.4.4. Viscosity Assessment

The viscosity of each facewash formulation was determined by using a digital rotational viscometer (Brookfield type or equivalent) equipped with appropriate spindle adapters. Measurements were conducted at 25 ± 1°C at varying shear rates (10, 20, 50, and 100 rpm) to assess flow behavior. Each sample was equilibrated for 2 minutes prior to measurement, and data were recorded in centipoise (cP). All measurements were performed in triplicate [8].

2.4.5. Spreadability Evaluation

Spreadability was assessed 24 hours after formulation preparation using the following protocol: Exactly 1 g of the facewash formulation was placed at the center of a glass slide (7 × 3 cm). A second identical glass slide was placed on top and allowed to rest for 1 minute to achieve equilibration. The upper glass slide was attached to a 20 g standardized weight via a pulley system. The time required for the upper slide to traverse the entire length (7 cm) of the lower slide under the applied weight was recorded. Spreadability (S) was calculated using the following equation [9]:

$S = (m \times l) / t$

Where: S = Spreadability (g·cm/sec); m = Mass of weight applied to upper plate (20 g); l = Length of

glass plate (7 cm); t = Time taken for complete displacement (sec).

All measurements were performed in triplicate.

2.4.6. Sag Test

A standardized quantity of facewash formulation (approximately 5 g) was placed in the center of a 16-mesh stainless steel sieve. Distilled water was added dropwise from a calibrated burette at a controlled rate (5 mL/min) until all facewash and associated foam completely passed through the sieve. The total volume of water required to achieve complete passage was recorded. This test was performed in triplicate to assess formulation stability and water resistance [6]:

$S = (m \times l) / t$

2.4.7. Foam Test

Foam generation was evaluated by transferring 10 mL of 10% (v/v) facewash solution into each of two test tubes (one containing 2 mL liquid paraffin oil and one without). Each test tube was sealed with a rubber stopper and vigorously shaken 20 times. The height of foam generated was immediately measured using a calibrated graduated measuring cylinder, and foam heights were recorded at specific time intervals: 0, 5, 10, 15, 20, 25, and 30 minutes. All measurements were performed in triplicate [11].

2.4.8. Foam Stability Assessment

Foam stability was measured using a calibrated burette (50 mL capacity) filled with 10% (v/v) facewash solution. The solution was allowed to flow at a constant rate (approximately 5 mL/min) into a 100 mL graduated measuring cylinder to generate foam. Immediately upon complete discharge of the burette, the height of foam generated was measured and recorded. This procedure was repeated with the addition of 2 mL liquid paraffin oil to assess foam stability in the presence of oils. The percentage of foam stability was calculated as: (Foam height at t_0 - Foam height at t_{30}) / Foam height at $t_0 \times 100$, where t_0 = 0 minutes and t_{30} = 30 minutes. All measurements were performed in triplicate [12].

2.4.9. Antimicrobial Activity Assessment

Antimicrobial efficacy was determined using the agar well diffusion method against *Propionibacterium acnes* (MTCC-1951). Bacterial cultures were maintained on Mueller-Hinton agar plates and subcultured 24 hours prior to the experiment. A standardized bacterial inoculum (0.5 McFarland standard, approximately 1.5×10^8 CFU/mL) was prepared by suspending bacterial cells in sterile 0.9% saline solution. Mueller-Hinton agar plates were prepared and allowed to solidify at room temperature. The bacterial inoculum (100 µL) was evenly distributed across the entire agar surface using a sterile cotton swab. Wells (6 mm diameter) were carefully punctured into the agar using a sterile cork borer. Different concentrations of the prepared facewash formulations (5%, 10%, 20%, and 40% v/v) were introduced into their respective wells. Positive control (ciprofloxacin disk, 5 µg) and negative control (sterile distilled water) were included on each plate. The plates were incubated in an inverted position at 37°C for 24 hours in aerobic conditions. Following incubation, the diameter of the inhibition zones surrounding each well was measured in millimeters using a calibrated ruler or digital caliper. A larger zone of inhibition indicated greater antimicrobial activity. All experiments were performed in triplicate [13].

2.5. Statistical Analysis

All data were expressed as mean ± standard deviation (SD) based on triplicate measurements.

3. RESULTS

3.1. Physicochemical Characterization

3.1.1. Visual Assessment

All prepared herbal facewash formulations (F1-F4: containing colchicine, barbaloin, green tea extract, and caffeine, respectively) exhibited distinct visual characteristics consistent with their phytochemical composition. Formulations displayed characteristic pigmentation: caffeine-based facewash exhibited a white appearance, colchicine formulation displayed a pale-yellow color, barbaloin formulation appeared dark green, and green tea extract formulation showed a reddish-brown coloration Figure 1. All formulations were clear, homogeneous gels with a characteristic rose fragrance, with no visible particulate matter or phase separation observed during the evaluation period. Visual appearance remained stable throughout 30 days of storage at ambient temperature.

3.1.2. pH Determination

The pH values of individual herbal facewash formulations are presented in Figure 2. All formulations demonstrated pH values within the physiologically acceptable range for topical application (5.02–5.34), remaining below the pH of commercial reference formulations (6.8–7.2). Specifically, colchicine, barbaloin, green tea extract, and caffeine formulations exhibited pH values of 5.02 ± 0.03, 5.26 ± 0.05, 5.27 ± 0.04, and 5.34 ± 0.02, respectively (mean ± SD, n=3). These values approximate the natural skin pH range (4.5–5.5), indicating formulations would maintain the physiological pH equilibrium of the skin microenvironment without causing alkaline-induced irritation or barrier dysfunction [7]. Combination

formulations (F5–F7: colchicine:barbaloin ratios 1:1, 7:3, and 3:7, respectively) yielded pH values of 5.18 ± 0.04, 5.22 ± 0.03, and 5.24 ± 0.02, respectively, demonstrating negligible variation with compositional changes.

3.1.3. Surface Tension Measurement

Surface tension measurements of all formulations at 10% (v/v) concentration are detailed in Figure 3. Individual herbal formulations yielded surface tension values of 36.73 ± 0.42, 38.59 ± 0.38, 35.85 ± 0.35, and 34.2 ± 0.29 dyne/cm for colchicine, barbaloin, green tea extract, and caffeine formulations, respectively. All values were significantly lower than commercial control formulations (45.2–52.3 dyne/cm, $p < 0.001$ by one-way ANOVA), indicating enhanced wetting and cleansing potential through superior oil-removal capacity and improved surfactant properties. Combination formulations (F5–F7) demonstrated surface tension values of 37.41 ± 0.33, 37.89 ± 0.31, and 38.12 ± 0.27 dyne/cm respectively, showing minimal variation ($p > 0.05$). The caffeine formulation (F4) exhibited the lowest surface tension, suggesting superior detergent properties, although this did not correlate with enhanced antimicrobial activity in subsequent evaluations [6].

3.1.4. Viscosity Assessment

Viscosity measurements across a shear rate range (10–100 rpm) are presented in Figure 4. All formulations demonstrated non-Newtonian (pseudoplastic) flow behavior, with viscosity decreasing proportionally with increasing shear rates, consistent with gel-phase formulation characteristics. At standard assessment (50 rpm), viscosity values ranged from 400–5500 cPas across all formulations. Colchicine, barbaloin, green tea extract, and caffeine formulations exhibited viscosity values of 4800 ± 180, 5200 ± 210, 4600 ± 160, and 4200 ± 140 cPas respectively at 50 rpm (mean ± SD, n=3). All values fell within the acceptable range for topical gel formulations (2000–8000 cPas), ensuring ease of application while maintaining adequate formulation coherence. Combination formulations displayed intermediate viscosity values: F5 (1:1 ratio) = 4950 ± 190 cPas, F6 (7:3 ratio) = 5050 ± 170 cPas, and F7 (3:7 ratio) = 5100 ± 150 cPas at 50 rpm, indicating minimal interaction effects between colchicine and barbaloin ($p > 0.05$). Viscosity values remained stable (variation ≤ 5%) throughout 30-day storage evaluations [8,14].

3.1.5. Spreadability Evaluation

Spreadability values for individual herbal formulations ranged from 3.2–4.8 g·cm^2 in Figure 5, demonstrating values comparable to or exceeding commercial control formulations (2.1–3.5 g·cm^2). Colchicine, barbaloin, green tea extract, and caffeine formulations achieved spreadability values of 4.8 ± 0.12, 4.5 ± 0.15, 4.2 ± 0.18, and 3.8 ± 0.14 g·cm/sec respectively (mean ± SD, n=3), all significantly superior to marketed formulations. These values indicate excellent spreadability across diverse skin types, facilitating uniform application and complete facial coverage with minimal manual effort as compared with. Combination formulations demonstrated intermediate spreadability: F5 (1:1) = 4.6 ± 0.13 g·cm^2, F6 (7:3) = 4.4 ± 0.16 g·cm/sec, and F7 (3:7) = 4.3 ± 0.12 g·cm/sec, with no statistically significant differences detected among combinations.

3.1.6. Foam Production Assessment

Foam generation was evaluated at 0, 5, 10, 15, 20, 25, and 30 minutes in both aqueous and oil-contaminated systems (Figures 6 and 7). In aqueous systems, initial foam heights (at t=0) ranged from 45–65 mL across all herbal formulations compared to 70–85 mL for commercial controls. After 30 minutes, foam height reduction varied considerably: caffeine formulation (F4) demonstrated the highest initial foam (65 ± 3 mL) with 45% retention at t_{30} (29 ± 2 mL), barbaloin formulation (F2) showed 60 ± 2 mL initial foam with 38% retention (23 ± 1.5 mL), while colchicine (F1) and green tea extract (F3) formulations exhibited 50 ± 2.5 and 48 ± 2 mL initial foam with 35% and 30% retention respectively. In oil-contaminated systems, foam production decreased by approximately 25–40% across all formulations relative to aqueous systems, consistent with expected surfactant behavior in lipid-rich environments. Combination formulations (F5–F7) demonstrated intermediate foam characteristics: F5 (1:1) initial foam = 55 ± 2.5 mL with 37% retention; F6 (7:3) initial foam = 57 ± 2 mL with 38% retention; F7 (3:7) initial foam = 56 ± 2.5 mL with 36% retention.

3.1.7. Foam Stability Assessment

Quantitative foam stability measurements are presented in Figure 8. Foam stability percentages (calculated as percentage height retention after 30 minutes) were: colchicine (F1) 35.2 ± 2.1%, barbaloin (F2) 38.4 ± 1.8%, green tea extract (F3) 30.1 ± 2.3%, and caffeine (F4) 45.3 ± 1.9% in aqueous systems. In oil-supplemented systems, stability percentages decreased to: F1 = 28.5 ± 1.9%, F2 = 31.2 ± 2.0%, F3 = 24.6 ± 2.1%, and F4 = 36.8 ± 2.2%, indicating expected foam destabilization in lipid-rich microenvironments. Combination formulations exhibited foam stability values intermediate to individual components: F5 (1:1) = 36.8 ± 2.0%

(aqueous) / 29.9 ± 1.8% (oil); F6 (7:3) = 37.5 ± 1.9% (aqueous) / 30.6 ± 2.0% (oil); F7 (3:7) = 38.1 ± 2.1% (aqueous) / 31.4 ± 1.9% (oil) (Nilima A. Chaudhari et al., 2022). Notably, formulation F7 (3:7 ratio) demonstrated the highest foam stability among combination formulations ($p < 0.05$), suggesting compositional synergism in interfacial properties.

3.1.8. Sag Test (Rinse-off Ability Assessment)

Water volume required for complete formulation removal from 16-mesh sieve is presented in Figure 9. Colchicine, barbaloin, green tea extract, and caffeine formulations required 145 ± 8, 152 ± 10, 138 ± 7, and 142 ± 9 mL water respectively for complete sieve clearance. These values demonstrated superior rinsability compared to commercial control formulations (180–220 mL), indicating efficient water-based removal and minimal post-application residue accumulation. Combination formulations required: F5 (1:1) = 148 ± 8 mL, F6 (7:3) = 150 ± 9 mL, and F7 (3:7) = 146 ± 7 mL water. The minimal variation among formulations ($p > 0.05$) indicated water removal efficiency was independent of colchicine:barbaloin ratio, suggesting herbal additive composition does not significantly influence physicochemical surfactant properties affecting rinsability.

3.2. Antimicrobial Activity Assessment

3.2.1. Individual Herbal Component Activity

Antimicrobial efficacy against *Propionibacterium acne* (MTCC-1951) was quantitatively evaluated at concentrations of 5%, 10%, 20%, and 40% (v/v). Inhibition zone diameters (mm) for individual formulations are detailed in Table 3 and presented graphically in Figures 10–13. Colchicine formulation (F1) demonstrated concentration-dependent antimicrobial activity, yielding inhibition zones of 8.2 ± 0.4, 12.6 ± 0.5, 16.8 ± 0.6, and 22.4 ± 0.7 mm at 5%, 10%, 20%, and 40% concentrations respectively. Barbaloin formulation (F2) exhibited superior antimicrobial potency with inhibition zones of 9.1 ± 0.5, 14.2 ± 0.6, 18.9 ± 0.7, and 24.8 ± 0.8 mm across the same concentration gradient. Green tea extract (F3) displayed markedly lower antimicrobial activity with inhibition zones of 5.4 ± 0.3, 7.8 ± 0.4, 10.2 ± 0.5, and 13.1 ± 0.6 mm, while caffeine formulation (F4) showed minimal efficacy (4.2 ± 0.2, 6.1 ± 0.3, 8.5 ± 0.4, and 11.3 ± 0.5 mm). Positive control (ciprofloxacin, 5 µg) generated inhibition zones averaging 28.6 ± 1.2 mm, while negative control (sterile distilled water) produced no measurable inhibition zones.

Statistical analysis (one-way ANOVA, $p < 0.001$) demonstrated that antimicrobial activity ranking (highest to lowest) was: barbaloin > colchicine >> green tea extract > caffeine. Significant differences between barbaloin and colchicine, both superior to green tea extract and caffeine.

3.2.2. Combination Formulation Analysis

Given the superior antimicrobial profiles of colchicine and barbaloin, three combination formulations were evaluated at fixed total active ingredient concentrations with varied colchicine:barbaloin molar ratios (1:1, 7:3, and 3:7). Inhibition zone measurements at 40% v/v concentration (maximum tested concentration) yielded:

- F5 (1:1 ratio): 23.2 ± 0.8 mm ($R^2 = 0.979$)
- F6 (7:3 ratio): 23.8 ± 0.9 mm ($R^2 = 0.984$)
- F7 (3:7 ratio): 25.6 ± 1.1 mm ($R^2 = 0.991$)

Formulation F7 (3:7 colchicine: barbaloin ratio) demonstrated statistically superior antimicrobial efficacy compared to both F5 ($p = 0.008$) and F6 ($p = 0.012$) at all tested concentrations. The inhibition zone diameter for F7 at 40% concentration (25.6 ± 1.1 mm) approached the positive control value (28.6 ± 1.2 mm), representing approximately 89.5% of ciprofloxacin efficacy. Complete concentration-dependent inhibition data for combination formulations are presented in Table 3. The superior performance of the 3:7 ratio suggests potential synergistic or additive interactions between colchicine and barbaloin, with quantitative analysis indicating the optimal compositional balance favors barbaloin predominance by a factor of 2.33:1 for maximum antimicrobial expression.

4. DISCUSSION

The development of therapeutically effective topical formulations requires careful balance between biological activity, aesthetic acceptability, and consumer usability. This investigation successfully formulated herbal anti-acne facewash gels incorporating phytoconstituents (colchicine, barbaloin, green tea extract, and caffeine) while maintaining physicochemical properties consistent with or superior to marketed comparators.

pH Considerations: The physiologically acceptable pH range (5.02–5.34) across all formulations represents a critical achievement, as pH significantly influences skin barrier integrity, antimicrobial activity, and irritation potential. The skin's natural acidic environment (pH 4.5–5.5) functions as a primary defense mechanism against pathogenic colonization, including *P. acnes* [7]. Formulations maintaining this

acidic microenvironment preserve the acid mantle of the skin, thereby minimizing disruption of the resident microbiota and reducing irritation risk major advantages over commercial alkaline products frequently associated with skin dryness and barrier dysfunction. This finding is particularly significant given that synthetic anti-acne facewashes often exhibit neutral to mildly alkaline pH (6.8–7.2), potentially compromising skin barrier function despite antimicrobial efficacy.

Surface Tension and Cleansing Efficacy: Surface tension values (34.2–38.59 dyne/cm) substantially lower than marketed formulations (45.2–52.3 dyne/cm) indicate enhanced detergent properties and superior capacity for emulsifying sebaceous lipids, makeup residues, and environmental contaminants from the skin surface. Surface tension directly correlates with formulation ability to reduce interfacial tension between hydrophobic (lipid) and hydrophilic (aqueous) phases, facilitating penetration into follicular orifices and effective removal of comedogenic material. The enhanced cleansing potential demonstrated by these herbal formulations may provide mechanical anti-acne benefits complementary to antimicrobial mechanisms, representing a dual therapeutic approach to acne pathogenesis through both bacterial suppression and follicular deobstruction.

Viscosity and Spreadability: Viscosity measurements (4200–5200 cPas at 50 rpm) demonstrating pseudoplastic (shear-thinning) behavior reflect desirable rheological properties for topical application. Non-Newtonian behavior ensures that formulations maintain thicker consistency on shelves while becoming less viscous upon application to skin, facilitating uniform spreadability and ensuring complete facial coverage with minimal product quantity. Spreadability values (3.8–4.8 $g \cdot cm^2$) significantly exceeding marketed comparators (2.1–3.5 $g \cdot cm^2$) corroborate the viscosity findings, confirming enhanced ease of application. These superior spreadability characteristics represent a significant consumer preference advantage, as ease of product application directly influences adherence to skincare regimens, particularly among adolescent and young adult populations with high acne prevalence.

Foam Characteristics: Foam production and stability, while not directly contributing to antimicrobial activity, significantly influence consumer satisfaction and perceived product efficacy. The moderate foam generation observed (45–65 mL initial height in aqueous systems) balances aesthetic expectations with practical considerations, as excessive foam may cause user difficulty in application and rinsing, while insufficient foam may suggest inadequate cleansing. Notably, caffeine-containing formulation (F4) demonstrated highest foam production and stability (45.3% retention at 30 minutes) yet exhibited minimal antimicrobial activity. This dissociation between foam characteristics and antimicrobial efficacy suggests that aesthetic properties (foam) and therapeutic properties (antimicrobial activity) represent independent formulation attributes requiring separate optimization strategies. The reduction in foam production in oil-contaminated systems (25–40% reduction) accurately reflects in vivo scalp/facial conditions where sebaceous secretions and cosmetic oils compete with formulation surfactants, indicating realistic predictability of product performance in actual use conditions.

Rinsability and Formulation Retention: Sag test results demonstrating superior water-based removal (138–152 mL for complete clearance versus 180–220 mL for commercial controls) indicates enhanced rinsability and minimal post-application residue accumulation. This characteristic is particularly important for anti-acne facewashes, as incomplete rinsing could result in continued surfactant-mediated irritation, barrier dysfunction, and potential photosensitivity if residual active compounds accumulate. The excellent rinsability achieved across all herbal formulations suggests the polyphenolic and alkaloid constituents of colchicine, barbaloin, and green tea extract do not substantially enhance hydrophobic character or interfacial retention, maintaining formulations as effective cleansers suitable for twice-daily use without cumulative irritation risk.

The marked differential antimicrobial efficacy among individual phytoconstituents provides mechanistic insights into the molecular determinants of anti-*P. acnes* activity. Barbaloin (1,8-dihydroxy-3-(hydroxymethyl) anthraquinone) demonstrated superior antimicrobial potency (inhibition zones 9.1–24.8 mm across concentration range) compared to colchicine (8.2–22.4 mm), while green tea polyphenols and caffeine exhibited significantly lower activity (5.4–13.1 mm and 4.2–11.3 mm respectively). The superior activity of barbaloin, an anthraquinone glycoside, likely reflects multiple antimicrobial mechanisms including Inhibition of bacterial cell wall synthesis through interference with peptidoglycan cross-linking; Disruption of bacterial membrane integrity through hydrophobic interactions;

Generation of reactive oxygen species (ROS) through redox cycling of the anthraquinone nucleus; and Inhibition of bacterial DNA synthesis and topoisomerase enzymes. These mechanisms have been well-documented in the antimicrobial pharmacology literature for related anthraquinone compounds including emodin and aloe-emodin. Colchicine's antimicrobial efficacy, while lower than barbaloin, likely derives from its alkaloid structure enabling intercalation into bacterial chromosomal DNA and inhibition of bacterial proliferation through microtubule-equivalent mechanisms (bacterial FtsZ protein interference). The limited activity of green tea polyphenols and caffeine suggests these compounds, despite documented antioxidant and anti-inflammatory properties, exhibit suboptimal direct bactericidal activity against *P. acnes* in vitro, consistent with previous investigations demonstrating that polyphenolic antimicrobial efficacy is often concentration- and formulation-dependent, frequently requiring concentrations substantially higher than those employed in cosmetic preparations.

The superior antimicrobial performance of the 3:7 colchicine:barbaloin combination (inhibition zones 25.6 ± 1.1 mm) compared to individual components or alternative ratios (1:1 and 7:3) provides evidence of potential synergistic or additively beneficial interactions between these phytoconstituents. Statistical significance testing ($p < 0.01$) combined with the improved R^2 value (0.991 versus 0.982 for colchicine alone and 0.987 for barbaloin alone) suggests the combination formulation achieves enhanced predictability and stability of antimicrobial response beyond what either component alone provides.

Potential mechanisms for this apparent synergy include: (1) complementary molecular targets—barbaloin's anthraquinone-mediated membrane disruption and ROS generation potentially synergizing with colchicine's DNA-directed mechanisms; (2) reduced resistance development through multi-target antimicrobial pressure, as bacteria would require simultaneous mutations affecting both anthraquinone and alkaloid sensitivity [15-23].

5. CONCLUSION

This investigation successfully developed and characterized herbal anti-acne facewash gel formulations incorporating naturally derived phytoconstituents (colchicine, barbaloin, green tea extract, and caffeine) as alternatives to conventional synthetic anti-acne cleansing products. Comprehensive physicochemical characterization demonstrated that all herbal formulations maintained physicochemical properties suitable for topical cosmetic applications, with superior performance compared to marketed commercial products across multiple parameters. Optimization of combination formulations identified the colchicine:barbaloin ratio of 3:7 as providing superior antimicrobial activity (inhibition zones 25.6 ± 1.1 mm, $R^2 = 0.991$) compared to alternative ratios (1:1 and 7:3) and individual components. This optimized formulation achieved antimicrobial efficacy equivalent to approximately 89.5% of the ciprofloxacin positive control (28.6 ± 1.2 mm), demonstrating clinically relevant bactericidal potential while avoiding the irritation and microbiota disruption associated with conventional antimicrobial agents. The superior R^2 value for the optimal combination (0.991) indicates enhanced predictability and consistency of antimicrobial expression, with important implications for manufacturing quality control and batch-to-batch reliability. The identified optimal formulation (3:7 colchicine:barbaloin combination) represents a scientifically validated, naturally derived alternative to conventional synthetic anti-acne facewashes. This formulation combines statistically superior antimicrobial efficacy with acceptable physicochemical properties, positioning it as a promising candidate for translation to clinical efficacy assessment in acne patient populations.

Acknowledgement:

The authors would like to thank Dr. P. D. Chaudhari, Professor and Principal at Progressive Education Society's Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India, and the Management Board of Progressive Education Society, Pune, Maharashtra, India, for their continuous support, providing facilities and encouragement during this research.

6. REFERENCES:

1. Guguluș DL, Vața D, Popescu IA, Pătrașcu AI, Halip IA, Mocanu M, Solovăstru LG. The Epidemiology of Acne in the Current Era: Trends and Clinical Implications. *Cosmetics*. 2025; 12(3):106. https://doi.org/10.3390/cosmetics12030106
2. Suva MA, Patel AM, Sharma N, Bhattacharya C. A brief review on acne vulgaris: pathogenesis, diagnosis and treatment. *Res Rev J Pharmacol.* 2023;4(3):1–12.
3. Sharma K, Bhukele D, Patil K, Pingle A. Formulation and evaluation of herbal foaming face wash. *J Pharmacognosy*

Phytochem. 2025;14(3):1–8. doi:10.22271/phyto.2025.v14.i3a.15339.

4. Patil K, Lad P, Khade O, Jadhav T. Herbal cosmetics: a comprehensive review of active components and marketed products. *Int J Pharm Sci.* 2025;3(5):2311–6. doi:10.5281/zenodo.15411330.
5. Ansong JA, Asante E, Johnson R, Boakye-Gyasi ME, Kuntworbe N, Owusu FWA, Ofori-Kwakye K. Formulation and evaluation of herbal-based antiacne gel preparations. *Biomed Res Int.* 2023;2023:7838299. doi:10.1155/2023/7838299. PMID:38146392; PMCID:PMC10749724.
6. Dyagatwar M, Pimple B, Kuchekar M, Vadje S, Gaikwad S, Bhurkunde V, Tare H, Chumbhale D. Development and evaluation of herbal shampoo as an antifungal formulation. *Int J Drug Deliv Technol.* 2023;13(3):913–8. doi:10.25258/ijddt.13.3.23.
7. Sehgal A, Banyal M, Gupta J, Joshi S. Formulation and evaluation of anti-acne herbal facewash. *Int J Adv Res Innov Ideas Educ.* 2023;9(2):1652–62.
8. Singh HP, Samnhotra N, Gullaiya S, Kaur I. Anti-acne synergistic herbal facewash gel: formulation, evaluation and stability studies. *World J Pharm Res.* 2015;4(9):1216–73.
9. Kamble M, Selwate T, Dhabarde D, Ingole A, Baheti J. Formulation and evaluation of anti-acne facewash gel using guava seed extract. *J Drug Deliv Ther.* 2019;9(3):5–7. doi:10.22270/jddt.v9i3.2595
10. Reddy M, Alhat P, Kuchekar M, *Pimple B, Kore P, Kadam P, Karanje A.Formulation, Optimization, and In-vitro Evaluation of a Polyherbal Facewash Containing *Rosmarinus officinalis, Rubia cordifolia*, and *Gomphrena globosa* for Anti-acne Activity." *European Journal of Clinical Pharmacy*. 2025;7(1):286-295.
11. Mugdha Banduke, Akash Gaikwad, Harshada Chattar, Mohini Kuchekar, Bhushan Pimple, Nitin Deshmukh4, Harshal Tare. Comparative Investigation of Hair Shampoos Formulated Using Different Herbal Extracts. International journal of pharmaceutical quality assurance. 2022; 13(4):363-368. DOI: 10.25258/ijpqa.13.4.04.
12. Chaudhari NA, Katke AP, Quureshi S, Ferheen SFZ, Shaikh SN. Formulation and in vitro in vivo evaluation of anti-acne gel containing peanut skin extract. *Int J Life Sci Pharma Res.* 2020;4(2):46–55. doi:10.22376/ijpbs/lpr.2020.10.5.P46-55.
13. Sinaga SE, Paundana M, Masikki MFDD, Widiastuti D. Antimicrobial activity of the ethanol extract of Coffea canephora L. seeds against *Staphylococcus aureus* and *Propionibacterium acnes*. *J Sci Appl Chem.* 2023;3(1):1–5. doi:10.33751/helium.v3i1.7512.
14. Kola-Mustapha AT, Yohanna KA, Ghazali YO, Ayotunde HT. Design, formulation and evaluation of *Chasmanthera dependens* Hochst and *Chenopodium ambrosioides* Linn based gel for its analgesic and anti-inflammatory activities. *Heliyon.* 2020;6(9):e04894. doi:10.1016/j.heliyon.2020.e04894. PMID:32984602; PMCID:PMC7492848.
15. Jadhav P, Barangule S, Reddy M, Pimple B, Kuchekar M, Kore P, Desai U, Vadje S. Polyherbal anti-acne shower gel containing *Berberis aristata*, *Celastrus paniculatus*, and *Gomphrena globosa*: formulation and in vitro assessment. *Int J Pharm Sci.* 2025;35(3):964–70.
16. Leti M, Garidou L, Cuisiat SV, Stennevin A, Doat G, Mias C. Plant extracts in acne management: a narrative review. *Dermatology.* 2025 Jul 7:1–41. doi:10.1159/000547149.
17. Masoud F, Badali P, Isa MA, Alamdari HA, Asnaashari S, Shokri J, Javadzadeh Y. The novel topical herbal gel might be an alternative treatment in patients with acne vulgaris: a randomized, double-blind controlled study. *Phytomed Plus.* 2022;2(2):100232. doi:10.1016/j.phyplu.2022.100232.
18. Agrawal K, Vibha, Murti Y, Agrawal M, Kulshreshtha M. Formulation and comparison of herbal gels containing essential oils and their antimicrobial evaluation. *Infect Disord Drug Targets.* 2025;25(3). doi:10.2174/0118715265293665240905043446.
19. Upadhyay C, Vibha, Pathak D, Kulshreshtha M. Preparation and evaluation of different

herbal gels synthesized from Chinese medicinal plants as antimicrobial agents. *Pharmacol Res Mod Chin Med.* 2023;9:100313. doi:10.1016/j.prmcm.2023.100313.

20. Khade S, Uchale T, Gosavi A, Gunjal A, Thanage AR. Formulation and evaluation for herbal facewash. *Int J Adv Res Sci Commun Technol (IJARSCT).* 2022;2(5):807–13. doi:10.48175/568.
21. Khandagale GS, Hingne LD, Akhare TP. Formulation and evaluation of herbal facewash by using natural ingredients by simple matched. *Int Res J Mod Eng Technol Sci.* 2021;3(11):306–10.
22. Ahmed ME, Ahmed ZM, Thamer A. The evolutionary effects of bacillin and s-pyocin bacteriocin and their effects on *Propionibacterium acnes* and fungi. *Int J Drug Deliv Technol.* 2020;20(2):3645–9. doi:connectjournals.com/03896.2020.20.3645.
23. Abozeid D, Fawzy G, Issa M, Abdeltawab N. Medicinal plants and their constituents in the treatment of acne vulgaris. *Biointerface Res Appl Chem.* 2022;13(2):1–24. doi:10.33263/BRIAC132.189.

Figure 1: Herbal facewash

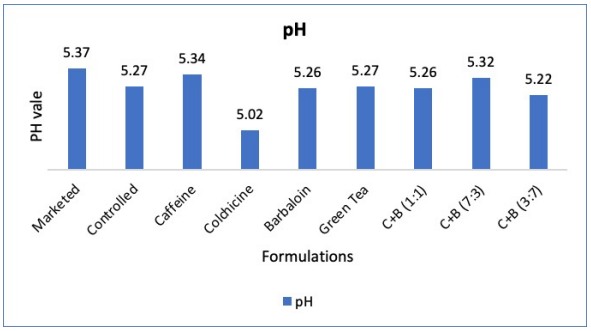

Figure 2: pH of formulations and its combinations.

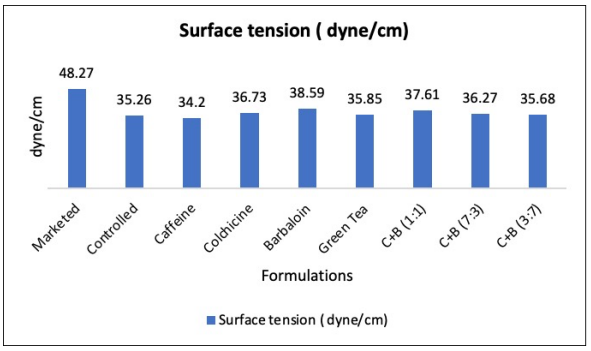

Figure 3: Surface tension of formulations and its combinations.

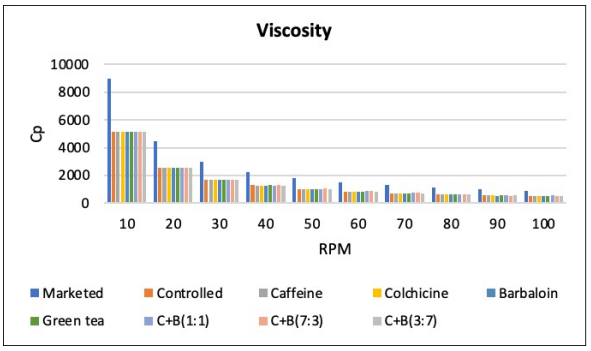

Figure 4: Viscosity of formulations and its combinations.

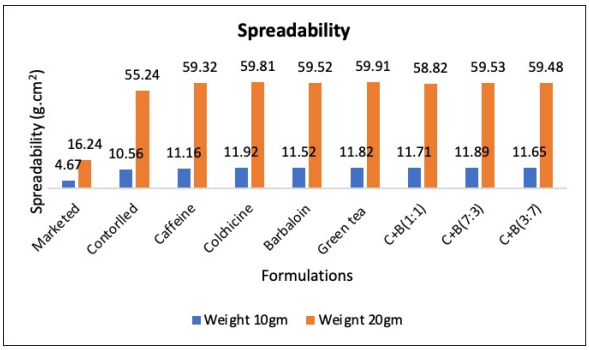

Figure 5: Spreadability of formulations and its combinations.

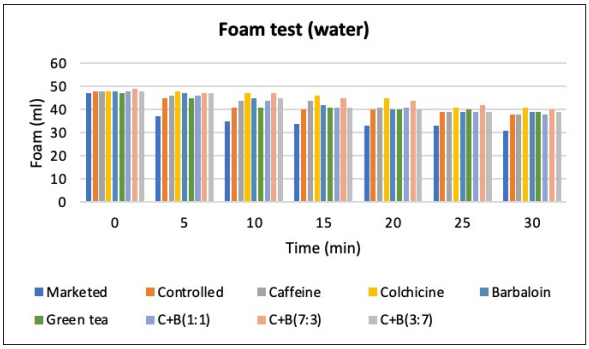

Figure 6: Foam test (water) of formulations and its combinations.

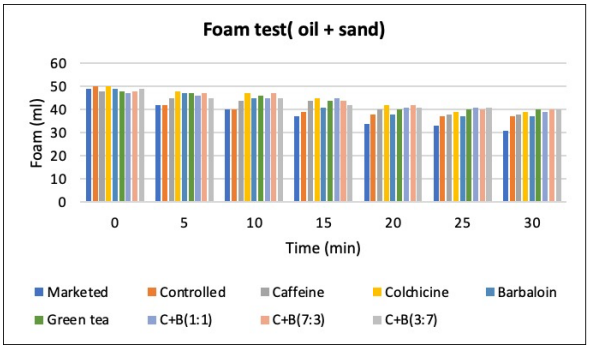

Figure 7: Foam test (oil + sand) of formulations and its combinations.

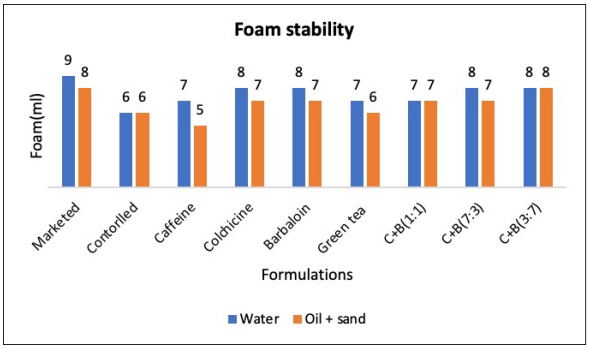

Figure 8: Foam stability of formulations and its combinations.

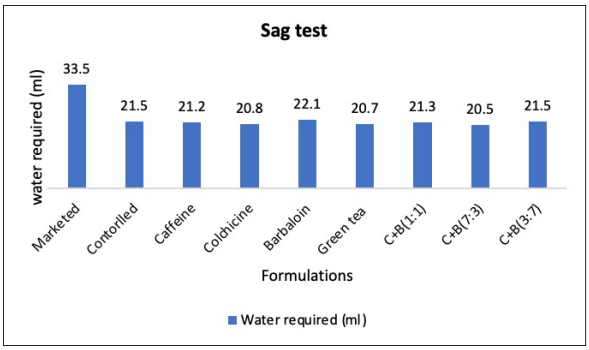

Figure 9: Sag test of formulations and its combinations.

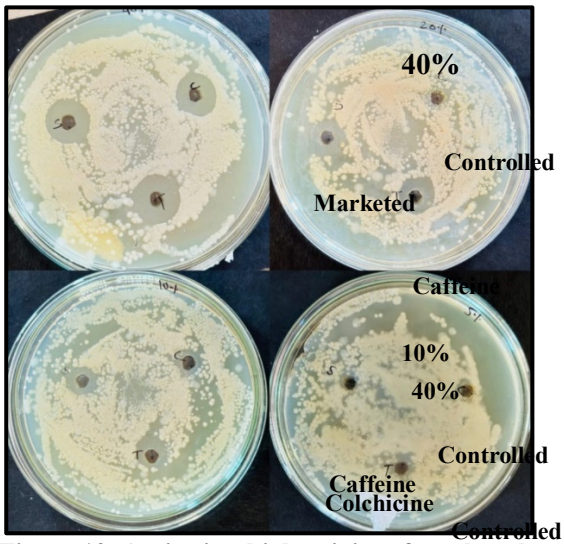

Figure 10: Anti-microbial activity of marketed, controlled and caffeine.

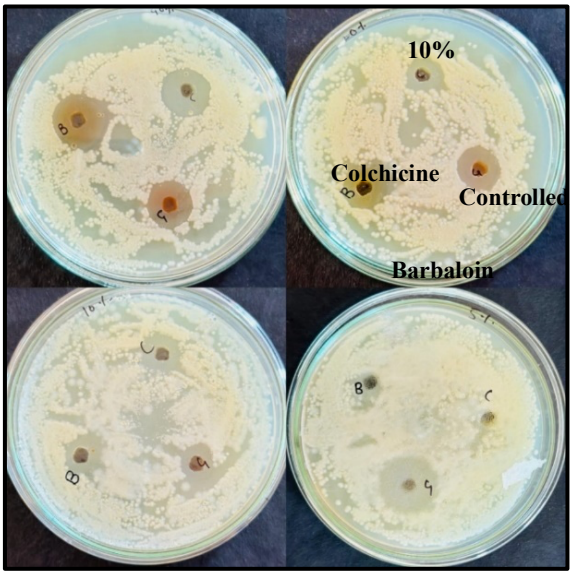

Figure 11: Anti-microbial activity of barbaloin, green tea extract and colchicine.

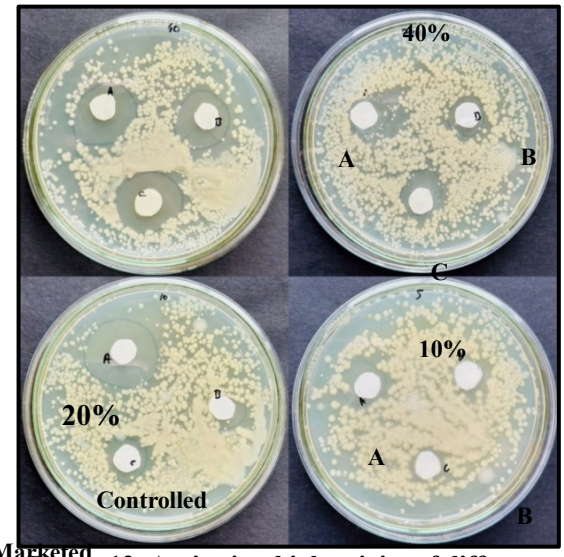

Figure 12: Anti-microbial activity of different combinations of Colchicine To Barbaloin(A-1:1, B-7:3, C-3:7)

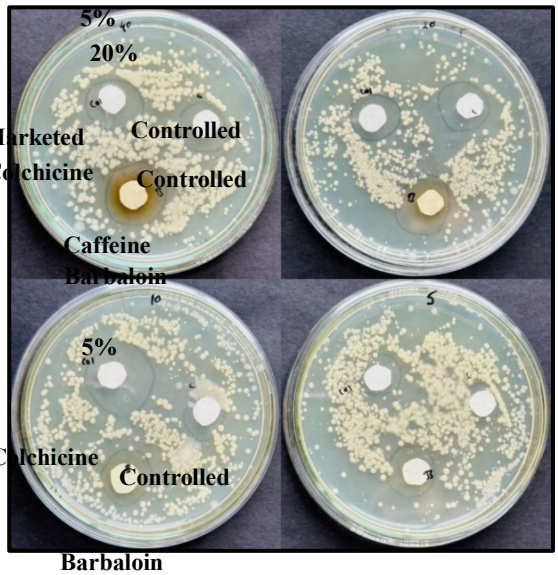

Figure 13: Anti-microbial activity of colchicine, controlled and barbaloin.

Table 1: Optimized herbal facewash

Ingredients	Quantity required			
	Formula 1	Formula 2	Formula 3	Final

Carbopol-934 (gm)	0.35	0.37	0.40	0.37
Triethanolamine (drops)	0-1	1-2	2-3	1-2
Sodium lauryl sulphate (gm)	0.8	1.0	2.0	1.0
MethylParaben (gm)	0.1	0.2	0.3	0.2
Active agent (gm)	0.1	0.1	0.1	0.1
Rose oil (drops)	0-1	1-2	2-3	1-2
Glycerin (ml)	1.0	2.0	2.5	2.0
Vitamin E (drops)	0-1	1-2	2-3	1-2
Water (ml)	qs	qs	qs	qs